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(54) Title: NEW DIAGNOSTIC ASSAY FOR DETECTION OF SYPHILIS		
(57) Abstract This invention relates to a novel assay for the diagnosis of syphilis using human serum or plasma. Syphilis is a sexually transmitted disease by the spirochaete micro-organism <i>Treponema pallidum</i> . Antibodies are produced in infected patients. Use of the invention results in a new and efficient means for detecting these antibodies, and hence infection. The invention provides a method for testing for the presence of antibodies to <i>Treponema</i> species in blood serum or plasma characterised by the addition of the following components to a reaction vessel in any sequence: a substantially undiluted sample of the test serum or plasma, erythrocytes coated with antigenic components of the target <i>Treponema</i> species, and reagents to neutralise the effects of antibodies to non- <i>Treponema</i> antigens or antibodies to <i>Treponema</i> species other than the target <i>Treponema</i> species mixing after the final addition and assessing agglutination of the erythrocytes, wherein the reaction vessel is coated with a binding agent which combats interaction between the vessel surface and the sample and/or erythrocytes causing false positive or false negative agglutination results.		

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1 "New Diagnostic Assay for Detection of Syphilis"

2

3 TECHNICAL FIELD

4

5 This invention relates to a novel assay for the
6 diagnosis of syphilis using human serum or plasma.

7 Syphilis is a sexually transmitted disease caused by
8 the spirochaete micro-organism *Treponema pallidum*.

9 Antibodies are produced in infected patients. Use of
10 the invention results in a new and efficient means for
11 detecting these antibodies, and hence infection.

12

13 BACKGROUND

14

15 Although Syphilis has been recognised as a specific
16 disease for over 500 years, it was not until the
17 beginning of this century that the causative organism,
18 *Treponema pallidum*, was first identified. The primary
19 means for detection of the disease was limited to
20 visual identification of the organism in human
21 syphilitic material. Syphilis is transmitted chiefly
22 by direct contact, the organism entering the body
23 through minute pores in the skin or mucous membranes.
24 In practice the most common route of infection is as a
25 sexually transmitted disease. Of particular concern is

1 that the disease may also be transmitted congenitally
2 from mother to foetus.

3
4 There are now available a number of techniques
5 available to diagnose syphilis in the infected
6 individual from a small sample of the patient's blood.
7 The pathogen can be directly visualised once there are
8 sufficient numbers of organisms in the sample, but
9 early diagnosis is hindered by the fact that *T.*
10 *pallidum* cannot be cultivated *in vitro* on artificial
11 media. Another common method is to use a non-
12 treponemal test; these tests use cardiolipin as the
13 active ingredient in a mixture with cholesterol and
14 lecithin to detect anti-cardiolipin antibodies found in
15 infected patients. Although providing a rapid method
16 for screening many samples, one of the problems with
17 these non-treponemal tests is the occurrence of both
18 false positives and false negatives.

19
20 Of inherently greater specificity are those tests which
21 detect the presence of anti-*T. pallidum* antibodies in
22 the patients blood. These use three main technologies,
23 ELISA, FTA-ABS and haemagglutination. ELISA tests have
24 been developed which use specific antigens bound to a
25 labelling enzyme. These offer good specificity but can
26 be expensive and time-consuming. Another very widely
27 used technique is the fluorescent *Treponema* antibody
28 absorption test (FTA-ABS), in which whole organisms are
29 fixed to glass slides and then overlaid with test
30 serum; the bound antibodies are detected with a
31 fluorescent anti-IgG conjugate. This method is very
32 specific but is laborious to carry out and hence is
33 more generally used as a confirmatory test rather than
34 a primary screen.

35
36 Haemagglutination is one of the most commonly used

1 screening tests. The test uses *Treponema* antigens
2 bound to red blood cells. The presence of anti-*T.*
3 *pallidum* antibodies in the test serum leads to
4 extensive cross-linking which results in agglutination
5 of the red blood cells to form a visible mat. These
6 are known generally as either *Treponema pallidum*
7 Haemagglutination Assays (TPHA) or Micro-
8 Haemagglutination Assay - *Treponema pallidum* (MHA-TP).
9 These assays are relatively cheap to produce and can be
10 automated by carrying out the reactions in microtitre
11 plates. In a typical TPHA the patients sample is
12 diluted in a suitable diluent and then a proportion of
13 this mixture is added to test cells (avian or ovine
14 erythrocytes coated with *T. pallidum* antigens). The
15 agglutination of the erythrocytes can be seen either
16 with the naked eye or by fully automated optical
17 methods.

18
19 One of the inherent problems of prior art TPHAs is the
20 requirement for the dilution step. The need for a
21 dilution step makes the assay harder to automate,
22 increases the time to complete the assay, increases the
23 cost of the procedure and limits the overall
24 sensitivity of the assay. Another disadvantage is the
25 potential loss of integrity of the patient's sample in
26 a two-stage process. The need for a dilution step
27 arises as a result of a number of non-specific binding
28 reactions which can occur between the test sample and
29 the antigens bound to the erythrocytes. The main
30 sources of these non-specific binding reactions are as
31 follows;

- 32
33 1. The *Treponema pallidum* organisms are grown in
34 rabbit testes. Subsequent purification of the
35 antigen is never completely effective with the
36 result that some rabbit antigens co-purify with

1 the *T. pallidum*. The presence in the test serum
2 of any anti-rabbit antibodies could lead to the
3 development of false positives.

4
5 2. The erythrocytes on which the *T. pallidum* antigens
6 are bound have antigenic sites themselves. Any
7 antibodies to these antigens present in the test
8 serum could also lead to the development of false
9 positives.

10
11 3. The *T. pallidum* antigens are not completely
12 specific to anti-*T. pallidum* antibodies; other
13 commonly found antibodies, particularly those
14 related to commonly found non-pathogenic related
15 species can react, so giving false positives.

16
17 A reliable assay for detection of *T. pallidum* antibodies
18 must therefore ensure that these three means of
19 interference are overcome, otherwise a significant
20 number of false positives will result. In prior art
21 TPHAs this is generally achieved by the addition of
22 neutralising substances to a diluent. The test sample
23 is then mixed with the diluent so neutralising the
24 various non-specific binding sites. Thus, the rabbit
25 antigens are neutralised by the addition of rabbit
26 serum and the erythrocyte antigens are neutralised
27 using a complex mixture of fragmented red blood cells
28 such as commercially available 'ox stroma'. The
29 reaction of non-*T. pallidum* antibodies with the *T.*
30 *pallidum* antigens is reduced by adding an autoclaved
31 mixture of related species of *Treponema*. Prior art
32 TPHAs have generally added these neutralising reagents
33 to a diluent. This results in two liquid handling
34 operations; addition of test sample to diluent and the
35 addition of a proportion of the diluent to the
36 activated red blood cells. This dilution step is also

1 thought to assist in reduction of non-specific binding
2 by diluting out contaminating antibodies.

3
4 Prior art attempts to remove the dilution step have
5 involved the addition of all the three neutralising
6 mixtures directly to the activated red blood cells.
7 These attempts have all failed as false positives are
8 not effectively screened out. A second problem is that
9 the positive agglutination patterns formed from genuine
10 positives collapse after a short period. There is
11 therefore a danger of both false positives and false
12 negatives. Therefore, no reliable single stage TPHA
13 for measurement of anti-*T. pallidum* antibodies exists.

14

15 STATEMENT OF INVENTION

16

17 It is the aim of the present invention to provide a
18 TPHA/MHA-TP which does not require a dilution step, but
19 nevertheless offers a highly specific diagnostic assay.

20

21 As used herein the term "substantially undiluted
22 sample" means a sample which is a test serum or plasma
23 in essentially the form in which it is taken from a
24 patient, in contrast to a sample diluted according to
25 the prior art TPHA/MHA-TP practices.

26

27 Many TPHAs, when used as a preliminary screen are
28 carried out in small reaction vessels such as
29 microtitre plates. Although automated liquid handling
30 systems can be used with microtitre plates, the
31 requirement for a dilution step adds significantly to
32 the complexity of the assay, with concomitant
33 repercussions on time, cost and sample integrity.

34

35 It has now been found that the hitherto insoluble
36 problem of prior art TPHAs, the requirement for a

1 dilution step, can be overcome by pre-coating the
2 surface of the reaction vessel with a binding agent.
3 This binding agent may react with reactive groups on
4 the surface of the reaction vessel to combat the cross-
5 linking of the sample or erythrocytes to the surface of
6 the reaction vessel leading to partial agglutination
7 (false positives) or interference with true positives
8 (false negatives). Using such pre-coated reaction
9 vessels it has been found that, surprisingly, the
10 neutralising reagents, the activated erythrocytes and
11 the test serum or plasma can be all mixed together
12 directly in the reaction vessel without the false
13 positives and false negatives which have previously
14 been seen.

15
16 The present invention thus enables a single stage TPHA
17 assay to be used to routinely screen large numbers of
18 samples which can be readily automated. The removal of
19 the dilution step has the added advantage that the
20 assay can also be made more sensitive, so enabling
21 earlier detection of syphilis.

22
23 In one embodiment of the invention a polystyrene
24 microtitre plate is used as the reaction vessel coated
25 with hydrolysed gelatin as the binding agent. Other
26 binding agents which have been found to be effective
27 include foetal calf serum, lactose, bovine serum
28 albumin, rabbit serum and casein digest. It has also
29 been found beneficial to mix certain of these binding
30 agents together, for example a mixture of hydrolysed
31 gelatin and lactose has been found to be a particularly
32 effective binding agent.

33
34 In another embodiment of the invention in order to
35 prevent the collapse of high titre positive samples,
36 the reaction vessel is coated with *T. pallidum*.

1 The invention can be used in a number of different
2 types of reaction vessel made of differing materials.
3 Thus microtitre plates, strip-well plates, cell culture
4 wells, cuvettes, test-tubes and the like can all be
5 coated with binding agent and used to carry out a
6 single stage TPHA assay. These reaction vessels can be
7 made of a number of materials including polystyrene,
8 polypropylene, polyvinyl chloride, polycarbonate,
9 polyethylene, terephthalate G copolymer or glass.

10
11 Although TPHAs have generally been carried out using
12 serum samples it has been found that a slight
13 modification allows efficient use of plasma samples.
14 Thus, in another embodiment of the invention it has
15 been found that when using fresh plasma samples the
16 single stage TPHA can be improved by the presence of
17 certain anti-coagulants, particularly heparin; such
18 anti-coagulant will usually be included in the test
19 cell mixture or formulation (the mixture of all other
20 components - coated erythrocytes, neutralising agents
21 etc - to which the test sample is added); its
22 concentration will generally be appreciably greater
23 than that convention for anti-coagulation purposes - eg
24 at least 340 units/ml of test cell formulation.

25

26 DETAILED DESCRIPTION OF THE INVENTION

27

28 The details of the invention will be described by way
29 of examples.

30

31 Example 1 shows the effects of using an uncoated
32 polystyrene microtitre plate in a single stage assay of
33 20 serum samples found to be negative for *T pallidum*
34 antibodies using a standard two stage TPHA. Of the 20
35 true negatives only 2 yielded a 'compact button'
36 indicative of a true negative with 1 indeterminate

1 result. This type of result would be clinically
2 unacceptable for unambiguous diagnosis of syphilis.

3
4 Example 2 shows the effect of coating the microtitre
5 plates with a number of different binding agents.
6 Although 2 show improvement over the uncoated plates,
7 hydrolysed gelatin gives the best improvement,
8 increasing the number of 'compact buttons' from 2 to
9 18, and thus improving the quality of the negative
10 patterns.

11
12 Example 3 shows that hydrolysed gelatin, Bovine Serum
13 Albumin (BSA) and Foetal Calf Serum (FCS) all give
14 promising results when mixed with Tween and lactose.
15 All coated plates show an improvement over the uncoated
16 plates.

17
18 Example 4 shows that the addition of sugars other than
19 lactose to the protein mixture gives good results,
20 though lactose is marginally better than the other
21 sugars tested.

22
23 In Example 5 plasma samples are used in place of the
24 serum samples tested previously. Surprisingly, the
25 results are not as good. This deficiency is readily
26 overcome by the addition of heparin at a concentration
27 of 340 - 1700 units/ml.

28
29 Example 6 shows that with a specimen containing a high
30 concentration of anti-*T. pallidum* antibody, collapse of
31 the agglutination pattern is seen unless the plate is
32 coated with *T. pallidum*.

33
34 In summary, it has been found that a variety of
35 substances coated onto the reaction vessel prevent the
36 formation of false positives and the collapse of high

1 titre positives, and so allow a single-stage TPHA to be
2 used as a screening method for both serum and plasma
3 samples. In a preferred embodiment a polystyrene
4 microtitre plate is coated with *T. pallidum*, 2% (w/w)
5 hydrolysed gelatin and 2% (w/w) lactose solution.

6
7 **EXAMPLES**

8
9 **EXAMPLE 1 UNCOATED MICROTITRE PLATES**

10
11 **Test Cell Formulation**

12
13 A formulation of Test Cells is made by mixing together
14 the following reagents:

15

16	Chicken erythrocytes coated with	0.4-0.85% w/w
17	<i>Treponema pallidum</i> antigen ¹	
18	Bovine serum albumin	5.0 mg/ml
19	Gentamycin sulphate	20 ug/ml
20	FTA sorbent	1.9% v/v
21	Sodium azide	1 mg/ml
22	Sodium chloride	16 mg/ml
23	Potassium and Sodium salts	7 mg/ml
24	Rabbit serum	0.4% v/v
25	Tween 80	0.1% v/v
26	Ox stroma	0.01% w/v
27	Distilled deionised water	

28

29 ¹Standard procedures are used to tan and coat the
30 erythrocytes with *T. pallidum* antigen (Tomizawa,
31 T. and Kasamatsu, S. Jap.J.Med.Sci.Biol.,
32 1966,19,305 and Sequiera, P.J.L. and Eldridge,
33 A.E. Brit. J. Vener. Dis., 1973,43,242.)

34
35
36

1 Specimens

2

3 20 fresh (maximum 2 days old) sera previously found to
4 be negative for syphilis antibody for a standard two
5 stage TPHA.

6

7 Procedure

8

9 10 μ l of serum are placed into a well of microtitrate
10 plate. 90 μ l of Test Cell Formulation are added and
11 the contents mixed by tapping or shaking the plate.
12 The plate is then incubated for one hour at room
13 temperature, and the plate examined either visually or
14 using an optical instrument.

15

16 Interpretation of Results

17

18 Agglutination of the cells is interpreted as a positive
19 result. Strong positives may show some folding at the
20 edge at the cell mat. Setting of the cells into a
21 compact button or a button with a pinprick hole in the
22 middle is interpreted as a negative result. Cells
23 showing partial agglutination resulting in a ring with
24 a large hole in the middle are interpreted as a +/-
25 indeterminate reaction.

26

27 Results

28

29 Agglutination Pattern	Number
30 Compact button	2
31 Button with pinprick	17
32 +/- indeterminate	1
33 False Postive	0

34

35

EXAMPLE 2 - MICROTITRE PLATES COATED WITH BINDING AGENT

A comparison of the effect of different structural types of binding agents bound to polystyrene microtitre plates is shown below.

Each of the binding agents is added to distilled water and sodium azide (0.1%w/v) is fully dissolved and mixed. 200 μ l of this solution is added to each well of a polystyrene microtitre plate. The plate is then incubated at 37°C, aspirated and dried. The Test Cell Formulation, Specimens and Procedure are as described in Example 1. The results shown are compared to the uncoated plate in Example 1.

Agglutination Pattern	Binding Agent			
	Uncoated	Hydrolysed gelatin 2% (w/v)	Lactose 2% (w/v)	Tween 20 0.01% (v/v)
Compact button	2	18	16	11
Button with pinprick	17	1	3	7
+/- indeterminate	1	1	1	1
False Positive	0	0	0	1

The results clearly show that two of the three types of binding agent have a beneficial effect. The hydrolysed gelatin has the most significant effect.

EXAMPLE 3 - EFFECT OF DIFFERENT PROTEIN MIXTURES AS BINDING AGENTS

Hydrolysed gelatin is one example of a complex protein

1 mixture. The data below show the effect of different
2 protein mixtures when combined with a sugar such as
3 lactose (2% (w/v)) and a surfactant such as Tween 20
4 (0.01% v/v). The Test Cell Formation, Procedure and
5 Specimens are as described in Example 1. The procedure
6 for coating the plates is as described in Example 2.

Agglutination Pattern	Binding Agent				
	Hydrolysed gelatin 2% (w/v)	BSA 1% (w/v)	FCS 2% (v/v)	RS 2% (v/v)	CD 2% (w/v)
Compact button	19	19	19	8	11
Button with pinprick	1	1	1	12	9
+/- indeterminate	0	0	0	0	0
False Positive	0	0	0	0	0

BSA - Bovine Serum Albumin
FCS - Foetal Calf Serum
RS - Rabbit Serum
CD - Casein digest

The results show that a number of protein mixtures,
when combined with sugar and surfactant, have a
beneficial effect over uncoated plates.

EXAMPLE 4 - EFFECT OF ADDITION OF DIFFERENT SUGARS TO HYDROLYSED GELATIN

The effect of adding alternative sugars with hydrolysed
gelatin is shown below. The procedure for coating the
plates is as described in Example 3, with the lactose
being replaced by other sugars.

1		Binding Agent			
2	Agglutination	Lactose	Maltose	Glucose	Dextran
3	Pattern				
4	Compact	19	18	18	15
5	button				
6	Button with	1	2	2	5
7	pinprick				
8	+/-	0	0	0	0
9	indeterminate				
10	Positive	0	0	0	0
11					

12 The results indicate that the addition of a number of
13 different sugars to a protein such as hydrolysed
14 gelatin has a beneficial effect, with lactose having
15 the greatest effect.

16
17 **EXAMPLE 5 - EFFECT OF ADDITION OF HEPARIN TO PLASMA**
18 **SAMPLES**

19
20 Specimens

21
22 20 fresh (maximum 2 days old) citrated plasma samples
23 previously found to be negative for syphilis antibody
24 by standard single stage TPHA.

25
26 All of the previous examples have demonstrated the
27 effectiveness of coating the reaction vessel with a
28 binding agent when using a serum sample. To test the
29 effectiveness of the binding agent with plasma samples
30 a polystyrene microtitre plate is coated with
31 hydrolysed gelatin, lactose, Tween 20 and azide as
32 described in Example 3. The results are not as good as
33 with serum. The addition of herapin at a concentration
34 of 340 - 1700 units/ml improves the results, comparable
35 with those achieved for the serum samples.
36

Agglutination Pattern	Without heparin	With heparin
Compact Button	15	19
Button with pinprick	3	1
+/- indeterminate	1	0
False Positive	1	0

EXAMPLE 6 - EFFECT OF *TREONEMA PALLIDUM* AS A BINDING AGENT

The effect of coating the plate with dilutions of *T. pallidum* prior to coating the plate with hydrolysed gelatin and lactose is shown in the data below. The *T. pallidum* (initial concentration 6.0×10^8 organisms/ml) is sonicated, diluted in saline and 100-200 μ l is added to each well of a polystyrene microtitre plate. The plate is incubated overnight at 4 C, aspirated and then the procedure for coating the plates is as described in Example 2.

Concentration of <i>T. pallidum</i>					
	0	1/3000	1/2000	1/1000	1/5000
Agglut-	collapse	partial	no	no	no
ination		collapse	collapse	collapse	collapse
pattern of					
high titre					
positive					

The results indicate that the addition of *T. pallidum* to the plate coat at a range of dilutions has a beneficial effect.

1 CLAIMS

2

3 1. A method for testing for the presence of
4 antibodies to *Treponema* species in blood serum or
5 plasma characterised by the addition of the following
6 components to a reaction vessel in any sequence:

7

8 a substantially undiluted sample of the test serum
9 or plasma,

10

11 erythrocytes coated with antigenic components of
12 the target *Treponema* species, and

13

14 reagents to neutralise the effects of antibodies
15 to non-*Treponema* antigens or antibodies to
16 *Teponema* species other than the target *Treponema*
17 species

18

19 mixing after the final addition and assessing
20 agglutination of the erythrocytes, wherein the reaction
21 vessel is coated with a binding agent which combats
22 interaction between the vessel surface and the sample
23 and/or erythrocytes causing false positive or false
24 negative agglutination results.

25

26 2. A method for testing for the presence of
27 antibodies to *Treponema* species in blood serum or
28 plasma which comprises pre-coating a reaction vessel
29 with binding agent which combats interaction between
30 the vessel and the sample and/or coated erythrocytes
31 causing false agglutination results and adding to the
32 reaction vessel in any sequence:-

33

34 erythrocytes coated with antigenic components of
35 the target *Treponema* species,

36

- 1 reagents which neutralise the effects of
2 antibodies to non-*Treponema* antigens or antibodies
3 to *Treponema* species other than the target
4 *Treponema* species, and a substantially undiluted
5 sample of the test serum or plasma
6
7 mixing after the final addition and assessing the
8 resulting agglutination pattern.
9
- 10 3. A means for testing for the presence of antibodies
11 to *Treponema* species in blood serum or plasma
12 characterised by the addition of the following
13 components to a reaction vessel in any sequence:
14
15 a substantially undiluted sample of the test serum
16 or plasma,
17
18 erythrocytes coated with antigenic components of
19 the target *Treponema* species, and
20
21 reagents to neutralise the effects of antibodies
22 to non-*Treponema* antigens or antibodies to
23 *Treponema* species other than the target *Treponema*
24 species
25
26 mixing after the final addition and assessing
27 agglutination of the erythrocytes, wherein the reaction
28 vessel is coated with a binding agent which combats
29 interaction between the vessel surface and the sample
30 and/or erythrocytes causing false positive or false
31 negative agglutination results.
32
- 33 4. A means or method according to any preceding claim
34 in which the binding agent contains at least one
35 component selected from proteins and sugars.
36

1 5. A means or method according to Claim 4 in which
2 the binding agent comprises at least one component
3 selected from the group consisting of hydrolysed
4 gelatin, bovine serum albumin, foetal calf serum,
5 rabbit serum, casein digest and lactose.
6

7 6. A means for testing for the presence of antibodies
8 to *Treponema* species in blood serum or plasma
9 comprising the addition of the following components to
10 a reaction vessel in any sequence:
11

12 a sample of the test serum or plasma,
13

14 erythrocytes coated with antigenic components of
15 the target *Treponema* species, and
16

17 reagents to neutralise the effects of antibodies
18 to non-*Treponema* antigens or antibodies to
19 *Treponema* species other than the target *Treponema*
20 species
21

22 mixing after the final addition and assessing
23 agglutination of the erythrocytes, characterised in
24 that the reaction vessel is coated with a binding agent
25 which comprises at least one component selected from
26 the group consisting of hydrolysed gelatin, bovine
27 serum albumin in combination with a surfactant and a
28 sugar, foetal calf serum, rabbit serum, casein digest
29 and lactose.
30

31 7. A means or method according to any preceding claim
32 in which the binding agent comprises bovine serum
33 albumin in combination with a surfactant and a sugar.
34

35 8. A means or method according to any preceding claim
36 in which the binding agent comprises bovine serum

1 albumin in combination with TWEEN® and a sugar.

2

3 9. A means or method according to any preceding claim
4 in which the binding agent comprises bovine serum
5 albumin in combination with TWEEN® and lactose.

6

7 10. A means or method according to Claims 4, 5, 6 or 7
8 in which the binding agent comprises both hydrolysed
9 gelatin and lactose.

10

11 11. A method for testing for the presence of
12 antibodies to *Treponema* species in blood serum or
13 plasma which comprises pre-coating a reaction vessel
14 with binding agent which comprises at least one
15 component selected from the group consisting of
16 hydrolysed gelatin, bovine serum albumin in combination
17 with a surfactant and a sugar, foetal calf serum,
18 rabbit serum, casein digest and lactose and then adding
19 to the reaction vessel in any sequence:

20

21 erythrocytes coated with antigenic components of
22 the target *Treponema* species,

23

24 reagents with neutralise the effects of antibodies
25 to non-*Treponema* antigens or antibodies to
26 *Treponema* species other than the target *Treponema*
27 species, and

28

29 a sample of the test serum or plasma

30

31 mixing after the final addition and assessing the
32 resulting agglutination pattern.

33

34 12. A diagnostic test kit for testing for the presence
35 of antibodies to *Treponema* species in blood serum or
36 plasma, the kit comprising the following components

1 erythrocytes coated with antigenic components of a
2 target *Treponema* species,

3

4 reagents to neutralise the effects of antibodies
5 to non-*Treponema* antigens or antibodies to
6 *Treponema* species other than the target *Treponema*
7 species, and

8

9 a reaction vessel

10

11 and wherein the reaction vessel is coated with binding
12 agent which combats interaction between the vessel and
13 one or both of [a] serum or plasma and [b] the coated
14 erythrocytes which would distort haemagglutination
15 assessment, wherein the binding agent is at least one
16 component selected from hydrolysed gelatin, bovine
17 serum albumin in combination with a surfactant and a
18 sugar, foetal calf serum, rabbit serum, casein digest
19 and lactose.

20

21 13. A diagnostic kit according to Claim 12 in which
22 the binding agent comprises bovine serum albumin in
23 combination with TWEEN® and a sugar.

24

25 14. A diagnostic kit according to Claims 12 or 13 in
26 which the binding agent comprises bovine serum albumin
27 in combination with TWEEN® and lactose.

28

29 15. A diagnostic kit according to Claim 12 in which
30 the binding agent comprises both hydrolysed gelatin and
31 lactose.

32

33 16. A means, diagnostic kit or method according to any
34 preceding claim in which the reaction vessel is a
35 microtitre plate, a strip-well plate, a cell culture
36 well, a test-tube or a cuvette.

- 1 17. A means, diagnostic kit or method according to any
2 preceding claim in which the reaction vessel is made of
3 polystyrene, polypropylene, polyvinyl chloride,
4 polycarbonate, polyethylene terephthalate G copolymer or
5 glass.
6
- 7 18. A means, diagnostic kit or method according to any
8 preceding claim in which the reaction vessel is a
9 polystyrene microtitre plate.
10
- 11 19. A means, diagnostic kit or method according to any
12 preceding claim in which the target *Treponema* species
13 is *Treponema pallidum*.
14
- 15 20. A means or method according to any preceding claim
16 in which the test sample is blood plasma and the
17 addition to the reaction vessel includes heparin.
18
- 19 21. A diagnostic kit according to any preceding claim
20 in which the components include heparin.
21
- 22 22. A means, diagnostic kit or method according to
23 Claims 20 or 21 wherein the heparin concentration is at
24 least 340 units/ml of the test cell formulation before
25 admixture with a test sample.
26
- 27 23. A means, diagnostic kit or method according to any
28 preceding claim in which *T. pallidum* is present as
29 binding agent.
30
- 31 24. A means, diagnostic kit or method according to any
32 preceding claim in which *T. pallidum*, hydrolysed
33 gelatin and lactose are present as binding agent.
34

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/01486

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/571 G01N33/555

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE WPI Section Ch, Week 7720, Derwent Publications Ltd., London, GB; Class B04, AN 77-35388Y & JP,A,52 044 229 (FUJIZOKI PHARM KK) 7 April 1977 see abstract</p>	1-24
A	<p>DATABASE WPI Section Ch, Week 9145, Derwent Publications Ltd., London, GB; Class B04, AN 91-328419 & JP,A,3 218 465 (SEKISUI CHEM IND KK) 26 September 1991 see abstract</p>	1-24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

21 October 1994

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Griffith, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/01486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 447 322 (SEKISUI CHEMICAL CO., LTD.) 18 September 1991 see page 5, line 26 - page 7, line 34 ---	1-24
A	EP,A,0 079 145 (FUJIZOKI PHARMACEUTICAL CO. LTD.) 18 May 1983 see the whole document ---	1-24
A	GB,A,1 577 131 (D. C. WHITLEY) 22 October 1980 see the whole document ---	1-24
A	EP,A,0 101 166 (KUJIREBIO KABUSHIKI KAISHA) 22 February 1984 see claim 7 -----	1-24

INTERNATIONAL SEARCH REPORT

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Int. Appl. No.
PCT/GB 94/01486

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